

EGFR-SEPT14 and *EGFR-PSPH* fusions is of interest because most of these events occurred in tumors that lacked the *EGFRvIII* rearrangement, which occurs in a large subset of glioblastomas¹⁴. Introducing *EGFR-SEPT14* into GSCs increased their proliferation, migration and activation of phosphorylated STAT3, an important signaling node for GSCs¹⁵ (Fig. 1b). Targeting the *EGFR-SEPT14* fusion protein with *EGFR* inhibitors such as lapatinib and erlotinib delayed tumor growth but only in tumors with *EGFR* genomic alterations. The complexity of *EGFR* mutations and amplifications poses challenges for therapeutic targeting; however, identifying fusion proteins may

permit targeting of the protein fused to *EGFR* (in this case, *SEPT14* or *PSPH*).

For personalized medicine to include the treatment of complex diseases such as cancer, therapies must truly be based on genomics. The first step in this process was undertaken by Frattini *et al.*¹, whose efforts led to the identification and validation of several new drivers for glioblastoma, which will hopefully expedite the development of new therapies.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Frattini, V. *et al.* *Nat. Genet.* **45**, 1141–1149 (2013).
2. Arikath, J. *et al.* *J. Neurosci.* **29**, 5435–5442 (2009).

3. Israely, I. *et al.* *Curr. Biol.* **14**, 1657–1663 (2004).
4. Jun, G. *et al.* *PLoS ONE* **7**, e43728 (2012).
5. Zeng, Y. *et al.* *Mol. Cancer* **8**, 19 (2009).
6. Dai, S.D. *et al.* *Cancer Sci.* **102**, 95–103 (2011).
7. Wang, M. *et al.* *BMC Cancer* **11**, 514 (2011).
8. Zhang, H. *et al.* *Target. Oncol.* doi:10.1007/s11523-013-0269-6 (20 February 2013).
9. Chauvet, N., Privat, A. & Prieto, M. *J. Comp. Neurol.* **479**, 15–29 (2004).
10. Korshunov, A. *et al.* *Acta Neuropathol.* **118**, 401–405 (2009).
11. Singh, D. *et al.* *Science* **337**, 1231–1235 (2012).
12. Parker, B.C. *et al.* *J. Clin. Invest.* **123**, 855–865 (2013).
13. Zheng, S. *et al.* *Genes Dev.* **27**, 1462–1472 (2013).
14. Dunn, G.P. *et al.* *Genes Dev.* **26**, 756–784 (2012).
15. Guryanova, O.A. *et al.* *Cancer Cell* **19**, 498–511 (2011).

Complex genetics of drug resistance in *Mycobacterium tuberculosis*

Digby F Warner & Valerie Mizrahi

Three new studies have used whole-genome sequencing of *M. tuberculosis* to demonstrate unexpected complexity in the modern evolution of drug-resistant tuberculosis, and a fourth study suggests a close evolutionary relationship between the pathogen and its human host over a period of 70,000 years. Collectively, the observations in these studies suggest that future strategies to tackle drug-resistant tuberculosis must integrate host genetics with detailed strain epidemiology.

Comparative genomic studies have identified fundamental aspects of the pathogenesis and evolution of *M. tuberculosis*, the causative agent of tuberculosis. However, deconvoluting complex virulence and drug resistance phenotypes remains challenging and is complicated by clinical classifications of *M. tuberculosis* strains as either susceptible or resistant, as these classifications rely on testing for a small number of defined resistance-associated mutations or on measured resistance to a single applied critical concentration of the drug. This binary classification system does not account for the range of drug susceptibilities present in strains¹. Now,

three separate studies in this issue describe the use of comparative analyses of whole-genome sequence data from clinical^{2,3} and laboratory⁴ *M. tuberculosis* isolates to identify mutations that might enable the emergence of drug resistance. In combination, these studies identify unexpected resistance-associated mutations and expand our understanding of the genetic diversity underlying drug resistance. Tuberculosis chemotherapy represents a recent selective force on *M. tuberculosis*, having been introduced approximately 70 years ago. To investigate the potential coevolution of *M. tuberculosis* with its obligate human host, a fourth study in this issue focused on the other end of the evolutionary timeline (Fig. 1), providing the context in which to consider the adaptation of modern strains to drug pressure.

Evolution of MTBC strains

Comas *et al.*⁵ sequenced and analyzed the genomes of 259 globally diverse strains of the *M. tuberculosis* complex (MTBC), a group of closely related mycobacteria that can cause tuberculosis. Their phylogenetic analysis suggested that MTBC strains emerged in Africa about 70,000 years ago. By comparing the evolutionary history of *M. tuberculosis* with

a corresponding analysis of ~5,000 human mitochondrial genomes representing the major human haplogroups, they inferred close coevolution of MTBC strains with anatomically modern humans as these humans migrated out of Africa and colonized different regions globally. The authors found that the abrupt increase in human density around 10,000 years ago, during the Neolithic Demographic Transition, was critical to the successful spread of this pathogen, pointing to human demography as a strong selective force. However, these results should be compared with those of another very recent study, which has argued against the codivergence of human and MTBC populations⁶.

Drug resistance

In one of the two papers using large collections of clinical *M. tuberculosis* isolates to identify genetic markers of drug resistance, Farhat *et al.*² report whole-genome sequencing of a panel of 123 *M. tuberculosis* strains that was chosen to ensure coverage of the major global *M. tuberculosis* lineages and to include progressively resistant isolates from community transmission chains or from individual patients with tuberculosis. Preferring not to employ commonly used tests to detect signatures of

Digby F. Warner and Valerie Mizrahi are in the Medical Research Council (MRC)–National Health Laboratory Service (NHLS)–University of Cape Town Molecular Mycobacteriology Research Unit and Department of Science and Technology (DST)–National Research Foundation (NRF) Centre of Excellence for Biomedical Tuberculosis Research, Institute of Infectious Disease and Molecular Medicine, and the Department of Clinical Laboratory Sciences, Faculty of Health Sciences, University of Cape Town, Rondebosch, South Africa.
e-mail: valerie.mizrahi@uct.ac.za

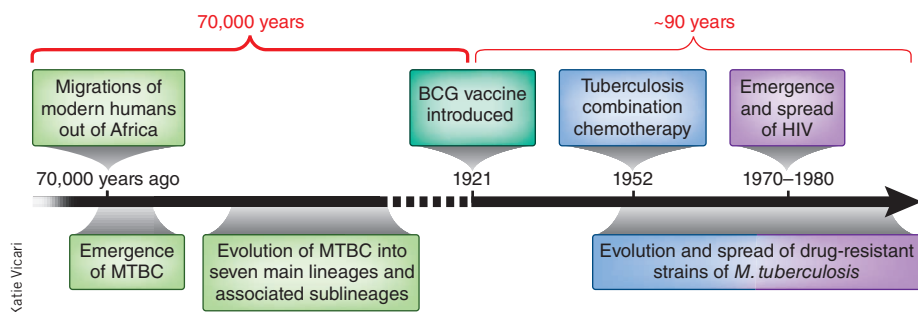


Figure 1 A timescale for the evolution of *M. tuberculosis* with its human host and the emergence of drug resistance. Four separate studies in this issue describe the use of comparative genomics to infer the selective pressures that have shaped the evolution of *M. tuberculosis* with its obligate human host⁵, as well as the adaptive mutations that might enable the emergence and fixation of drug resistance in clinical *M. tuberculosis* strains^{2,3}, including resistance to ethambutol⁴.

selective pressure, on the grounds that the clonal expansion underlying *M. tuberculosis* diversity precludes the useful application of these tests, the authors instead employed a new phylogenetic convergence test that identified all 11 known resistance determinants and 39 new targets of independent mutation (TIMs) located in 30 genes and intergenic regions (IGRs). A sizeable number of TIMs mapped to the large family of PE/PPE genes that encode proteins whose precise role in mycobacterial pathogenesis remains obscure⁷. Five other TIMs were located in genes involved in cell wall biosynthesis or remodeling. Importantly, the authors experimentally confirmed the functional impact on altered drug susceptibility of a specific mutation in one of these genes, *ponA1*.

In the second study, Zhang *et al.*³ searched for the genes, IGRs and nonsynonymous SNPs most strongly linked to resistance in a panel of 161 *M. tuberculosis* strains from China, almost all of which were from lineage 2 (mainly from East Asia) or lineage 4 (mainly from Europe). Their initial analysis, restricted to multidrug-resistant (MDR) and extensively multidrug-resistant (XDR) isolates, identified many known resistance-associated genes. When applying this analysis to the full panel of strains, they identified a further 70 genes (including 3 well-known drug resistance-associated genes) and 19 IGRs associated with resistance to the major first- and second-line tuberculosis drugs. Using the ratios of nonsynonymous to synonymous SNPs (dN/dS ratios) to measure the impact of selection, the authors found that antibiotic pressure has exerted a small but positive selective effect on the *M. tuberculosis* genome, with sites showing signs of such positive selection, for this particular collection at least, mapping almost entirely to the 84 drug resistance-associated genes identified in the study.

These two studies^{6,7} identify candidate genes associated with resistance to one or more drugs

that warrant further investigation and show a tantalizing association of IGRs with drug resistance, which is consistent with the growing appreciation of the potential role of non-genic regions in mycobacterial physiology⁸. These studies also detected resistance-associated mutations that were present in drug-sensitive strains, suggesting that these mutations might represent early events in the sequential acquisition of resistance or, alternatively, might confer an incremental fitness advantage. Similarly, separate analyses of drug-resistant isolates confirmed that extensive multidrug resistance arises through the accumulation of nonsynonymous mutations that are associated with resistance to second-line drugs rather than as a result of mutations in a small number of genes conferring pan-drug resistance. The studies also highlight the relevance of cell wall remodeling pathways to drug resistance, an emerging theme that echoes recent observations from related work⁹. There were some notable absences of known mutations, too, including in *rpsA*—recently proposed to underlie an elusive alternative mechanism of resistance to the frontline tuberculosis agent pyrazinamide¹⁰—with these mutations not identified as associated with resistance in either study.

In the third study, Safi *et al.*⁴ applied a combination of *in vitro* selection and elegant molecular genetic techniques to gain new insight into the evolution of ethambutol resistance in *M. tuberculosis*. Importantly, this work suggests that the acquisition of high-level ethambutol resistance occurs in a multistep process. According to the model of Safi *et al.*, mutations in *embB* are followed by mutations in pathways for the biosynthesis (*Rv3806c*) or use (*Rv3792*) of the cell wall precursor decaprenylphosphoryl- β -D-arabinose (DPA), which, finally, are followed by mutations in *embC*. In *Rv3792*, Safi *et al.*⁴ provide the first

example of an association between a synonymous SNP and drug resistance in *M. tuberculosis*. Moreover, the *Rv3806c* (UbiA) protein functions in tandem with another enzyme, DprE1, which has emerged as a promiscuous target of new anti-tuberculosis agents¹¹. Consistent with the presence of DprE1 and *Rv3806c* in the same biochemical pathway, ethambutol does not appear to act synergistically with known DprE1 inhibitors¹²; however, it might be interesting to consider whether *dprE1* mutants are associated with differential susceptibility to ethambutol. Finally, it is notable that mutations in *Rv3806c* were identified separately *in vitro*⁴ and in clinical isolates³, but, in contrast to the demonstrated role of this gene in ethambutol resistance, it was implicated in fluoroquinolone resistance in Zhang *et al.*³. A similar disconnect is also suggested by the discrepant *Rv3806c*-associated phenotypes reported by Safi *et al.*⁴ and in previous work by the same group¹³.

Tuberculosis treatment and control

Together, the papers highlighted here expand the panel of *M. tuberculosis* drug resistance markers and suggest that, although prevalent, low-level resistance might remain undetected by current diagnostic methods. These findings have significant implications for global tuberculosis control efforts and reinforce recent proposals to replace current clinical microbiology methods for routine diagnosis and drug susceptibility testing with high-throughput whole-genome sequencing approaches¹⁴. In addition, the expanded genetic profile of resistance may be useful for clinical management and therapeutic selection. These possibilities have special significance in regions of the world, such as southern Africa, where tuberculosis continues to exact a devastating toll.

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1. Böttger, E.C. *Clin. Microbiol. Infect.* **17**, 1128–1134 (2011).
2. Farhat, M.R. *et al. Nat. Genet.* **45**, 1183–1189 (2013).
3. Zhang, H. *et al. Nat. Genet.* **45**, 1255–1260 (2013).
4. Safi, H. *et al. Nat. Genet.* **45**, 1190–1197 (2013).
5. Comas, I. *et al. Nat. Genet.* **45**, 1176–1182 (2013).
6. Pepperell, C.S. *et al. PLoS Pathog.* **9**, e1003543 (2013).
7. Sampson, S.L. *Clin. Dev. Immunol.* **2011**, 497203 (2011).
8. Zhang, Y.J. *et al. PLoS Pathog.* **8**, e1002946 (2012).
9. Sun, G. *et al. J. Infect. Dis.* **206**, 1724–1733 (2012).
10. Shi, W. *et al. Science* **333**, 1630–1632 (2011).
11. Zumla, A., Nahid, P. & Cole, S.T. *Nat. Rev. Drug Discov.* **12**, 388–404 (2013).
12. Lechartier, B., Hartkoorn, R.C. & Cole, S.T. *Antimicrob. Agents Chemother.* **56**, 5790–5793 (2012).
13. Motiwala, A.S. *et al. J. Infect. Dis.* **201**, 881–888 (2010).
14. Köser, C.U. *et al. N. Engl. J. Med.* **369**, 290–292 (2013).